

Temperature affects physiological stress responses to acute confinement in sunshine bass (*Morone chrysops* × *Morone saxatilis*)

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Received 26 May 2004; received in revised form 17 September 2004; accepted 19 September 2004

Abstract

Sunshine bass (*Morone chrysops* × *Morone saxatilis*) were subjected to a 15-min low-water confinement stressor at temperatures ranging from 5 to 30 °C. Physiological responses were evaluated by measuring hematocrit, and plasma chloride, glucose and cortisol. Fish acclimated to 30 °C had initial glucose concentrations of 3.13 mM (564 mg/L) which were significantly lower than in fish acclimated to 5 and 10 °C (4.32 and 4.82 mM or 779 and 868 mg/L, respectively). Fish survived the conditions imposed at every temperature except 30 °C, where 15 out of 42 fish died during the stress and recovery protocol. The general pattern was an initial increase in hematocrit, followed by a delayed decrease in hematocrit and chloride, and an increase in plasma glucose and cortisol. In general, fish stressed at temperatures below 20 °C had lower and more delayed changes in plasma glucose and cortisol than fish tested at 20, 25 and 30 °C. Initial cortisol concentrations were 65 ng/ml and increased to above 200 ng/ml in fish held at 20 °C and above. At the higher temperatures, glucose concentrations were twice the initial concentration after stress and cortisol changes were four to five times the initial concentration after the stress. Quantitative responses for glucose and cortisol were moderate and recovery rapid in fish stressed at 10 and 15 °C; therefore, this range of water temperature is recommended when handling sunshine bass.

Published by Elsevier Inc.

Keywords: Cortisol; Glucose; Chloride; Confinement; Stress; Sunshine bass; Temperature

1. Introduction

Sunshine bass are hybrids produced by mating female white bass (*Morone chrysops*) with male striped bass (*Morone saxatilis*). This hybrid is the most common of the *Morone* hybrids produced for aquaculture. Juvenile striped bass demonstrate maximum growth at about 24 °C (Cox and Coutant, 1981). Studies on growth and temperature interaction in hybrids yielded optimal growth temperatures 3–8 °C higher (Woiwode and Adelman, 1984, 1991), although these studies were done with palmetto bass, which are a cross of female striped bass and male white bass.

Many aquaculture practices, including moving fish (Davis et al., 1993) and confinement (Davis and Parker,

1990), induce physiological alterations in response to the stress caused by the disturbances. Physiological stress is a non-specific response composed of a primary neuroendocrine component characterized by sympathetic activation and the secretion of epinephrine and cortisol (Donaldson, 1981; Schreck, 2000). A secondary phase is characterized by an increase in plasma glucose and osmoregulatory disturbances. Cortisol is a steroid hormone with many biological activities (Mommensen et al., 1999) including gluconeogenesis (Freeman and Idler, 1973) and immunosuppression (Schreck, 1996). The degree of the stress experienced is related to the severity and longevity of the stressor, and is reflected in physiological responses including cortisol and glucose (Carmichael et al., 1984a,b). Further, the ambient water temperature also dramatically changes the response to stress and the recovery dynamics. It is commonly observed that fish (Hodson and Hayes, 1989) tolerate handling better when the water temperature is low.

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Lower water temperature has several advantages. Cooler water holds more dissolved oxygen, pathogen populations do not reproduce as rapidly and the general metabolic rate of the fish is diminished. A temperature that would limit the magnitude of the stress response and still support a rapid recovery of the physiological alterations should identify an optimum temperature for handling fish to lower mortality. Such a temperature is expected to be different for different species and likely is affected by the thermal optimum for other metabolic activities such as growth. This study was undertaken to determine physiological stress responses in sunshine bass acclimated to a wide range of temperatures to help determine the temperature range at which sunshine bass can be most effectively handled.

2. Materials and methods

Sunshine bass were produced at Keo Fish Farm (Keo, AR, USA) and raised at the Harry K. Dupree-Stuttgart National Aquaculture Research Center until they were about 18 months old. Fish weighed 191.7 ± 57.8 g ($n=24$) at the beginning of the study and were in the range of fish referred to as phase II fingerlings (Hodson and Hayes, 1989). For each temperature tested, six fish were stocked into each of seven 60-l aquaria provided with aeration provided by airstones, and flowing well water at 23 °C. Experimental temperatures were achieved at a rate of change of 2 °C per day by passing the water through a gas heater or a cooling coil. When the desired temperature was reached, fish were held for 5 days before an experiment was conducted. After completion of each experiment, the water temperature was either raised or lowered to the next experimental temperature. Groups of fish to be tested at temperatures below 23 °C were stocked at the same time. All fish were exposed to 2 days of temperature adjustment and then held for 5 days at 20 °C before the experiment was conducted. Remaining fish were exposed to 3 days of temperature adjustment to 15 °C and 5 days of acclimation and tested at that temperature. A similar pattern of temperature adjustment and acclimation period was followed for fish tested at 10 and 5 °C. The same pattern was followed for fish tested at 25 and 30 °C. Each fish was sampled only once during the entire experiment.

At the beginning of each experiment, an initial group of six fish was sampled and fish in remaining tanks were exposed to an acute confinement stressor by turning off the

water and replacing the standpipe in each tank with a shorter one, thereby lowering the water level from 60 to 5 l within 5 min. The water level was selected so that the fish were submerged but were unable to maintain their posture in the tank. The second group of 6 fish was sampled 15 min after removal of the tall standpipe. Water flow was restored, the tall standpipe was replaced, and the third sample taken 1 h after replacing the standpipe. Complete restoration of the 60-l volume required 45 min. Subsequent groups of six fish each from separate tanks were taken at 2, 6, 24 and 48 h after replacing the standpipes.

Sampling was done rapidly, without anesthesia, by three individuals to avoid changes during the sampling period. Sampling was usually completed in 3 min. Blood was collected in heparinized syringes from the caudal vessels in the hemal arch. A sample of whole blood was collected in micro-hematocrit capillary tubes and centrifuged. Remaining blood plasma was separated by centrifugation in 12×75 mm culture tubes and stored frozen for later analysis. Plasma chloride was measured with a Corning 925 chloride analyzer (Bayer, East Walpole, MA, USA). Plasma glucose concentrations were determined by the glucose oxidase procedure (Sigma Diagnostics, No. 510A, St. Louis, MO, USA). Plasma cortisol was determined by radioimmunoassay (RIA) using the BioChem Immunosystems Cortisol Bridge kit (No. 14394, Polymedco, Cortlandt Manor, NY, USA) which has been validated for sunshine bass (Davis and Griffin, 2004).

Comparisons among sampling times at each temperature were done by analysis of variance followed by Tukey's multiple range test when statistical significance ($P<0.05$) was indicated. The same procedure was used to test differences among the initial samples at each temperature.

3. Results

Acclimation temperature significantly affected the initial concentrations of plasma chloride and glucose. Chloride was significantly higher in fish acclimated to 5 °C than in those acclimated to 25 °C. Glucose concentrations in fish held at 30 °C were significantly lower than fish held at 5 or 10 °C. The high variation of plasma cortisol obscured any difference in initial concentrations due to temperature (Table 1).

Table 1
Initial blood characteristic (means±S.E., $n=6$) of sunshine bass acclimated to six different temperatures

	Acclimation temperature (°C)					
	5	10	15	20	25	30
Hematocrit (%)	27.8±2.6	37.3±2.2	29.3±3.8	37.3±5.9	31.5±2.5	34.7±1.8
Chloride (mmol/l)	134.7±1.6 ^a	124.2±2.7 ^{a,b}	125.8±4.2 ^{a,b}	129.6±5.3 ^{a,b}	115.3±4.8 ^b	125.2±2.2 ^{a,b}
Glucose (mg/dL)	77.9±5.0 ^{a,b}	86.8±7.3 ^a	63.3±6.2 ^{b,c}	63.3±2.8 ^{b,c}	69.6±3.0 ^{a,b,c}	56.4±1.1 ^c
Cortisol (ng/ml)	12.5±5.7	33.8±13.1	36.1±17.1	65.4±9.3	49.9±20.5	20.0±4.2

Means with the same letter are not significantly different ($P<0.05$).

The low-water confinement stress did not affect survival except for those fish tested at 30 °C; In fish tested at 30 °C, 15 of the 42 exposed fish died after cessation of the stressor.

Blood hematocrit did not change at any time in fish held at 5 or 15 °C and was significantly lower after 48 h of recovery in fish held at 10 °C. In fish tested at 20 and 25 °C,

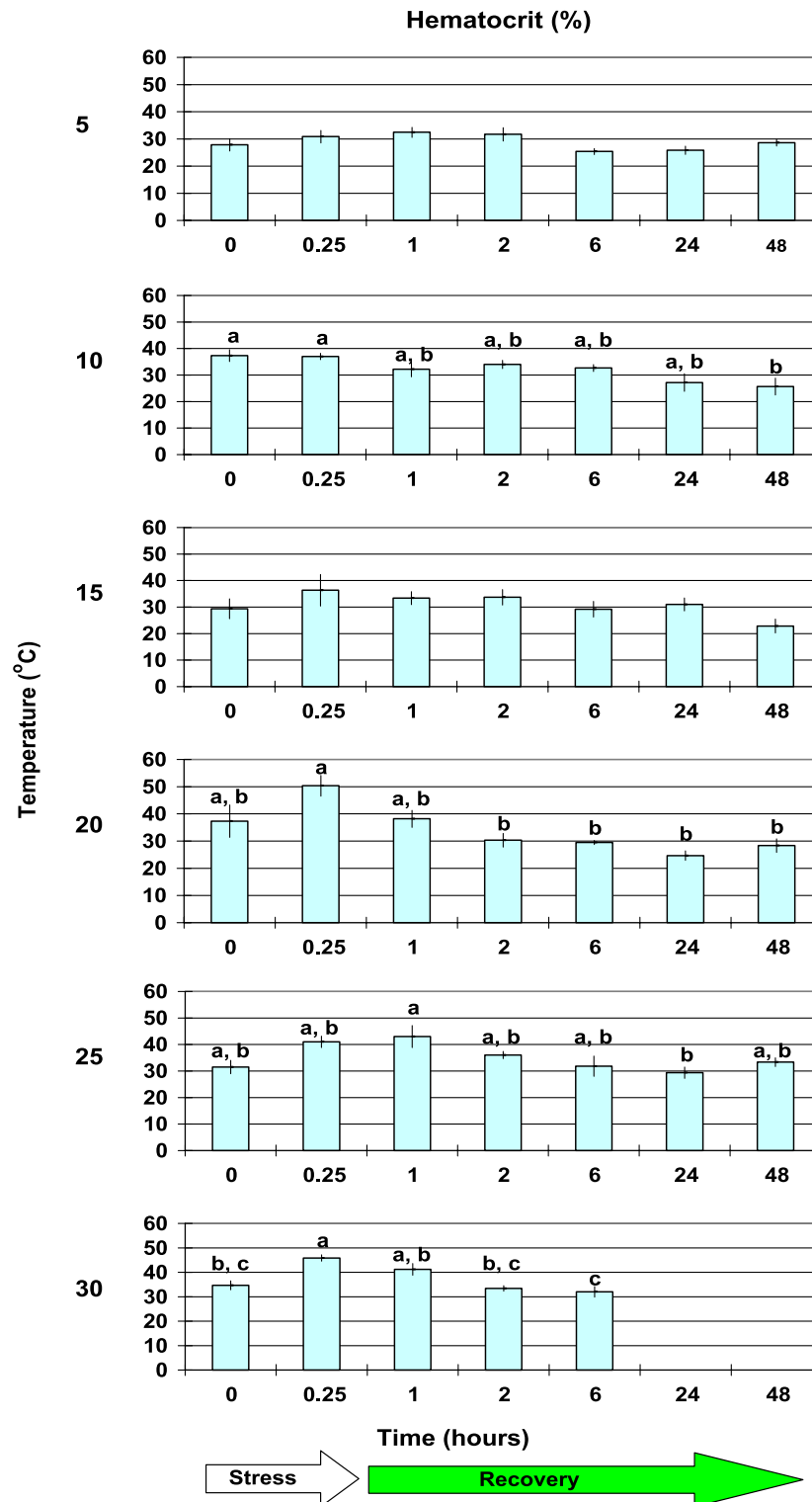


Fig. 1. Hematocrit changes during and following a low-water confinement stressor in sunshine bass acclimated to different temperatures. Values are the means \pm SE before disturbance (0), after 15 min of low-water confinement (0.25), and 1, 2, 6, 24 and 48 h after restoring the water volume to the tank. Data are the means of six fish in most cases. Missing data represent treatments where no fish survived. Different letters by each mean represent statistically different subsets ($P < 0.05$).

no significant differences in hematocrit were observed from the initial value. In fish tested at 30 °C, the only significant change in hematocrit was an increase after the 15-min confinement stressor (Fig. 1).

Plasma chloride did not change at any time in fish tested at 5 °C and was significantly lower after 48 h of

recovery in fish tested at 10 °C. At 15 °C, plasma chloride remained unchanged from the initial value. Plasma chloride concentrations were lower than initial levels in fish tested at 20 °C after 6 h of recovery and after 2 h in fish tested at 30 °C. No significant changes occurred in fish held at 25 °C (Fig. 2).

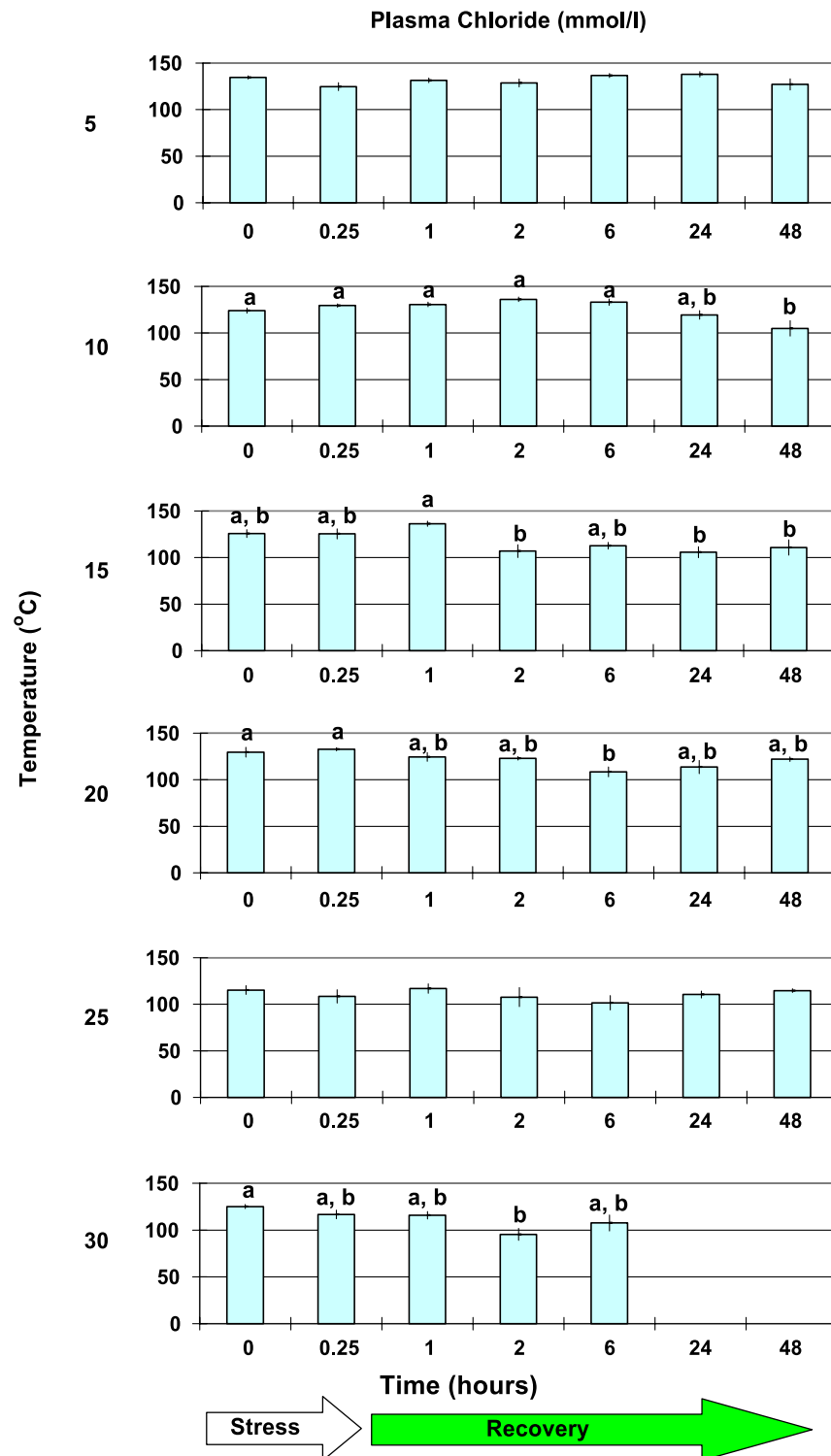


Fig. 2. Plasma chloride concentration changes during and following a low-water confinement stressor in sunshine bass acclimated to different temperatures. Experimental details are the same as those described in Fig. 1.

Plasma glucose in fish held at 5 °C was higher than the initial concentration in unstressed fish after the 15-min confinement stressor and after 2 h of recovery, but was not significantly different from the initial group at any other time during recovery. No significant changes in plasma glucose occurred at any time in fish held at 10 or 15 °C.

Plasma glucose in fish held at 20 °C was significantly higher than that of the initial group after the 15-min confinement stressor and remained so through the 2-h recovery, after which concentrations were not different from the initial group. When fish were held at 25 °C, plasma glucose was significantly higher than the initial

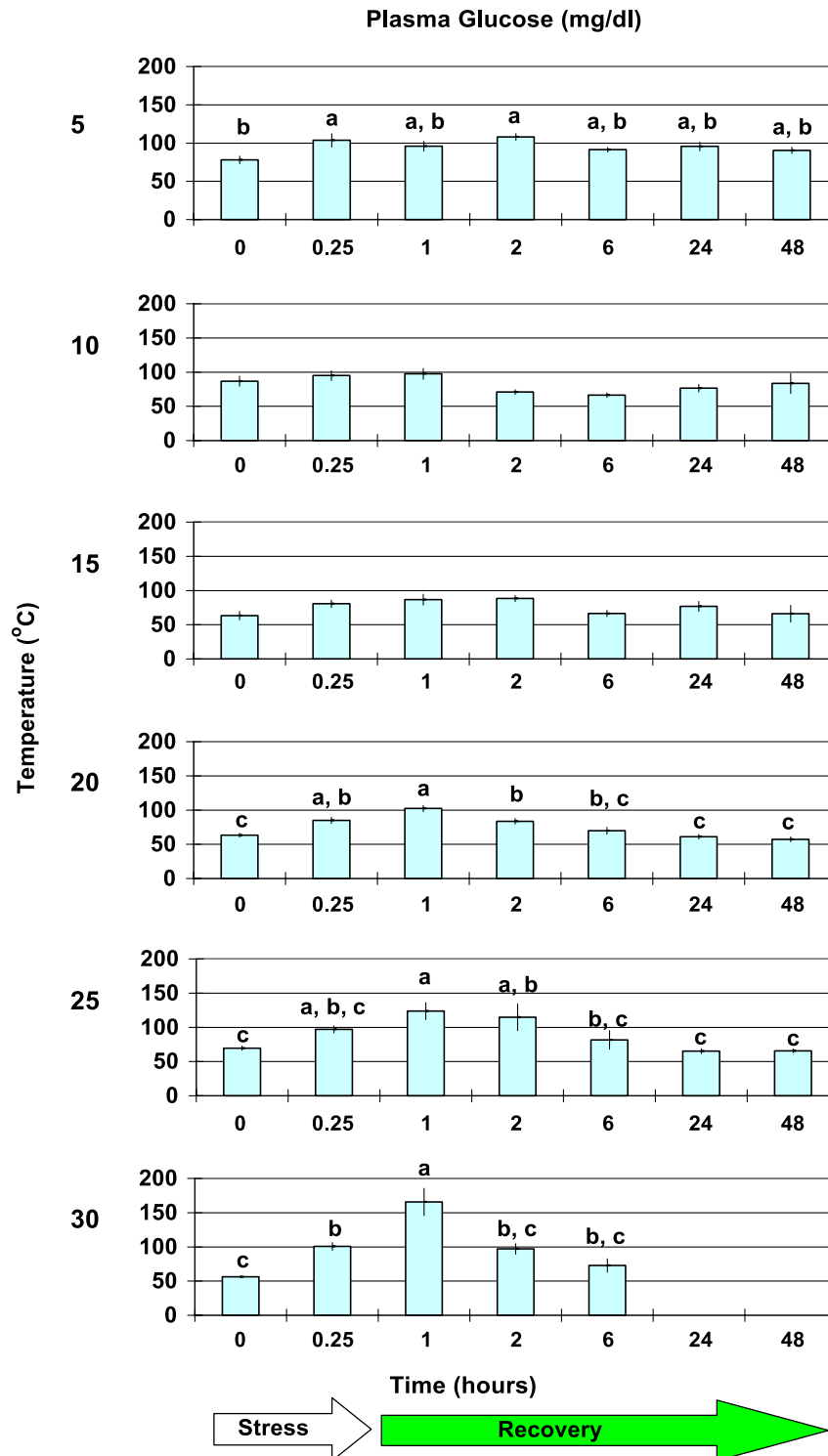


Fig. 3. Plasma glucose concentration changes during and following a low-water confinement stressor in sunshine bass acclimated to different temperatures. Experimental details are the same as those described in Fig. 1.

group at 1 and 2 h of recovery, then returned to initial levels. Plasma glucose was significantly increased by 15 min of confinement in fish stressed at 30 °C, increased further after 1 h of recovery, then returned to initial levels in the remaining fish (Fig. 3).

After the 15-min low-water confinement stressor in fish tested at 5 °C, a significant increase in plasma cortisol was not apparent until 1, 2 and 6 h after the water level was restored. Recovery to initial levels was complete by 24 h after the water level was restored. A

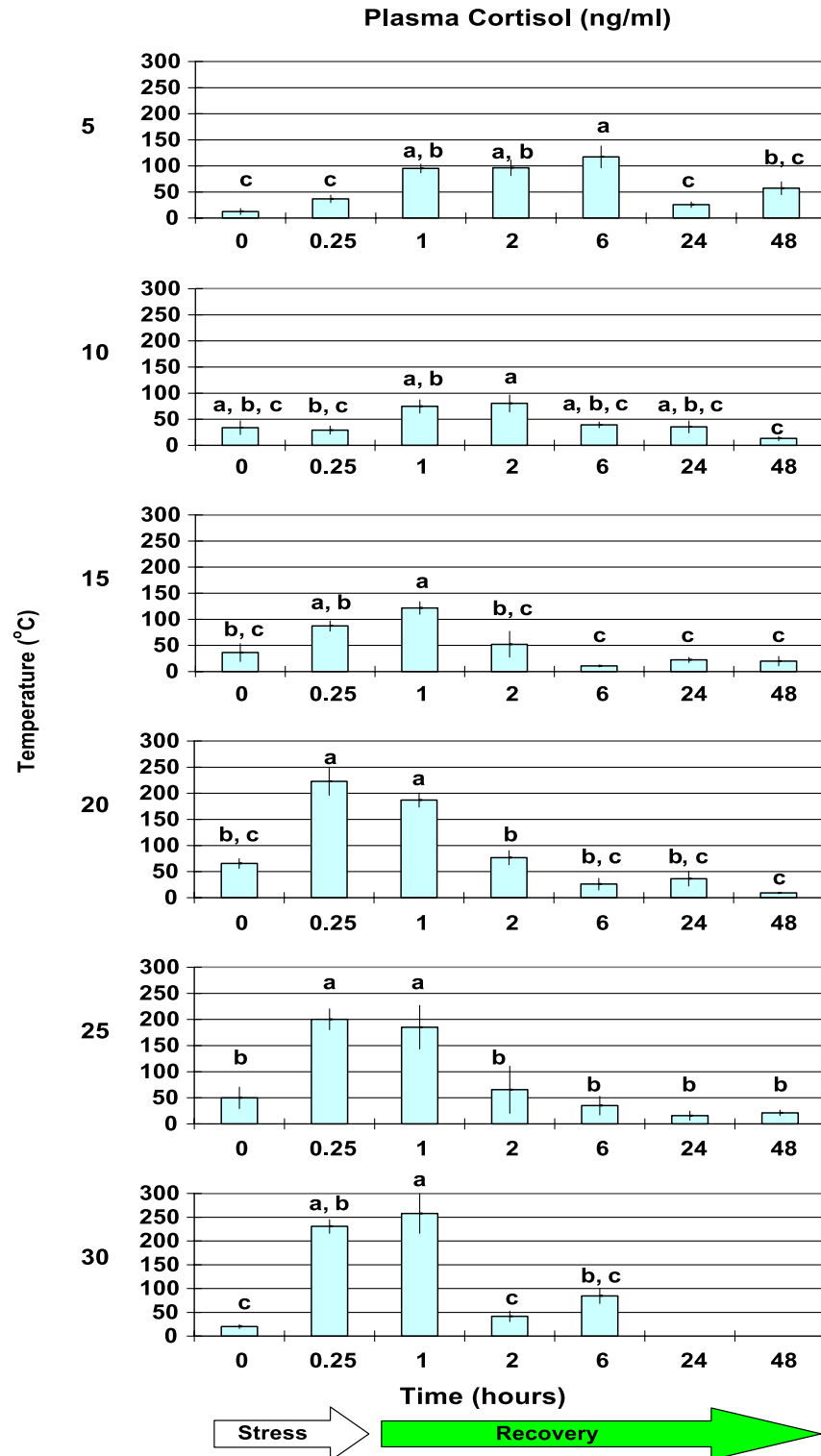


Fig. 4. Plasma cortisol concentration changes during and following a low-water confinement stressor in sunshine bass acclimated to different temperatures. Experimental details are the same as those described in Fig. 1.

similar response appeared to occur in fish tested at 10 and 15 °C, however, the increase from the initial concentrations was not consistent enough to be significant in fish tested at 10 °C. Fish tested at 15 °C had significantly higher cortisol concentrations 1 h after the water level was restored and had returned to initial levels by 2 h. Plasma cortisol was significantly higher than initial levels in fish tested at 20, 25 and 30 °C after the 15-min low-water confinement and remained higher 1 h after recovery. After 2 h of recovery, plasma cortisol concentrations were not different from concentrations in fish at the beginning of the experiment. The response pattern was similar in fish tested at the three highest temperatures. A significant increase above the initial concentrations occurred after 15 min of confinement and remained high for 1 h during the recovery period. By 2 h of recovery, cortisol concentrations were similar to the initial levels and remained so until 48 h. Fifteen fish held at 30 °C died following the confinement stressor (Fig. 4).

4. Discussion

Acclimation temperature had a much less dramatic effect on blood constituents of the initial sample of sunshine bass than those reported for striped bass (Davis and Parker, 1990). All of the plasma characteristics measured had significantly different values due to the acclimation temperature in striped bass and the values for glucose and cortisol were particularly affected. Plasma glucose was significantly lower in both sunshine bass and striped bass at the highest temperature, but the magnitude of the effect of the acclimation temperature was much greater in striped bass. Plasma cortisol concentrations in striped bass acclimated at 30 °C were much higher than at any other temperature and remained higher for a longer time than the concentrations in fish stressed at any other temperature. A temperature effect in resting concentrations of cortisol in channel catfish (*Ictalurus punctatus*) has been reported where fish acclimated to low temperature had significantly higher cortisol concentrations than fish acclimated to higher temperatures (Strange, 1980; Davis et al., 1984). In Davis and Parker (1990), the striped bass were not fed for 14 days prior to the stress test while sunshine bass in the present study were offered a maintenance ration of about 1% of the body weight per day until the day the stress test was performed. While a difference due to nutrition cannot be ruled out, the lack of an effect on resting concentrations in sunshine bass might suggest that these fish can better tolerate a wider range of temperatures than striped bass.

Fish appeared to recover quickly after the 15-min confinement and were swimming normally by 2 h of recovery in all groups except those held at 30 °C. The effect of confinement on the fish at 30 °C appeared harsh as 15 fish died during the first 6 h of recovery.

The general pattern of change in hematocrit was an initial increase at the end of the 15-min stress followed by return to pre-stress levels within 2 h, but this pattern was significant only in fish tested at 30 °C and was not observed in fish held at 5 or 10 °C.

A similar delay in the decrease of plasma chloride following a stressor has been reported in largemouth bass (*Micropterus salmoides*) (Carmichael et al., 1984a,b). Lower temperatures may have delayed the decrease in plasma chloride and the recovery to pre-stress concentrations. A lack of a significant change in fish tested at 25 °C may suggest that ion regulation and other metabolic events may be more stable at that temperature and are altered less by stressful conditions.

The general pattern for the response of plasma glucose was also affected by the temperature. A low, transient increase was observed at 5 °C and no significant change occurred at 10 or 15 °C. From 20 through 30 °C, the increase in glucose increased with temperature.

The response of plasma cortisol due to the confinement stress was also temperature dependent. Plasma cortisol increased dramatically and remained elevated for 1 h after recovery in fish tested at the three highest temperatures, then returned to initial levels after 2 h. Responses at the three lower temperatures were quantitatively lower and sometimes delayed. A significant increase in fish tested at 15 °C occurred 1 h after recovery. Similar temperature-dependent effects on cortisol secretion have been reported in channel catfish (Davis et al., 1984). The changes in plasma cortisol at the lower temperatures were likely caused by a combination of lower synthesis and secretion of cortisol due to lower metabolic activity.

Conditions that result in minimal changes of these physiological characteristics might be expected to be those that least upset the homeostatic state of the fish. Any alteration of plasma electrolytes likely involves metabolic activity by all the cells to restore osmoregulatory balance. The elevation of plasma glucose is likely due to mobilization of glucose from glycogen in the liver and is probably caused by adrenalin secretion and general sympathetic activation (Mazeaud and Mazeaud, 1981). The loss of glucose can deplete carbohydrate energy stores and cortisol is known to induce gluconeogenesis (Vijayan et al., 1996). Gluconeogenesis results in converting non-carbohydrates, proteins and fats, into glucose (Freeman and Idler, 1973). Increased circulating cortisol has also been linked to immunosuppression (Maule et al., 1989). This is especially true for stressors that induce chronic elevations of cortisol. Recent evidence suggests, however, that modest, acute increases in cortisol may increase immune function and disease resistance in fish (Maule et al., 1989) and mammals (Dhabhar and McEwen, 2001). A complete elimination of the cortisol stress response might be therefore detrimental to the fish.

The data in this study indicate that stress-induced changes of all the blood components tested were least in sunshine bass acclimated to 10 or 15 °C. Fish acclimated to 5 °C apparently

had lost some of their metabolic regulatory ability since changes were minor to absent. This might indicate a reduced ability to react to environmental changes. Palmetto bass have maximum growth at 27–29 °C (Woiwode and Adelman, 1984), about 3–8° higher than striped bass (Cox and Coutant, 1981). Stress-inducing activities during aquaculture must take into account several practical issues: how long the fish are to be handled, water quality characteristics (especially temperature), and the characteristics of the water they are to be transferred into. Changing water temperature can be expensive, requires time for acclimation and increases handling time. Handling fish at water temperatures that allow moderate physiological adjustments and avoid extreme stress responses might result in better survival of the fish. It seems reasonable that optimum temperatures for fish to tolerate disturbances would be lower than optimum temperatures for growth and food conversion. Considering these requirements, the optimum temperature for handling sunshine bass is between 10 and 15 °C. This range is the same that was recommended for striped bass (Davis and Parker, 1990). Clearly, handling sunshine bass at temperatures above 25 °C should be avoided if possible. The effects of the lower temperatures are a result of a combination of a decreased metabolic rate of the fish which decreases the magnitude of all of the stress responses.

Acknowledgements

Mention of a trade name, proprietary product or specific equipment in the article is solely for the purpose of providing information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. The gift of the sunshine bass by Keo Fish Farm is gratefully acknowledged. The author thanks Matthew McEntire and Jan Franks for excellent technical support and Drs. Bill A. Simco, Richard Spieler, and David Straus for review of the manuscript.

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